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## MiniReview

# Phytochemistry, pharmacology and toxicology properties of *Strobilanthes crispus*

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**Abstract**

*Strobilanthes crispus* (*S. crispus*) is an herbal medicine plant which is native to countries from Madagascar to Indonesia. The plants contained high amount of mineral content and vitamin C, B1 and B2. This plant are used in medicinal and to treat a variety of ailments in the various traditional systems of medicine. Phytochemical investigations have revealed that the plant contain polyphenols, flavonoids, catechins, alkaloids, caffeine, tannins, compounds known to possess multiple health beneficial effects. Preclinical studies have shown that the plant possess antioxidant, free radical scavenging, anticancer, antidiabetic, antimicrobial, wound healing and antiulcerogenic activities. This review presents the comprehensive overview of phytochemical constituents, pharmacological and toxicological properties of *S. crispus* and to provide preliminary information for future research and for commercial exploitation.



## Introduction

*Strobilanthes crispus* (L.) Bremek or *Saricocalyx crispus* (L.) Bremek (Acanthaceae) is native to countries from Madagascar to Indonesia (Sunarto, 1977). This plant is locally known as daun "picah beling" in Jakarta or "enyoh kelo" or "kecibeling" or "kejibeling" or "ngokilo" in Java (Sunarto, 1977) and also "pecah beling" or "bayam karang" or "pecah kaca" or "jin batu" in Malaysia (Noraida, 2005). This bush-like plant is attaining a maximum height 0.5-1.0 m (Figure 1). It can be found on riverbanks or abandoned fields (Noraida, 2005). The leaves are oblong-lanceolate, rather obtuse and shallowly crenate crispate and have rough surface, covered with short hairs (Backer and Bakhuizen, 1963; Sunarto, 1977). The upper surface of the leaves is darker green in colour and less rough as compared to underside (Sunarto, 1977) (Figure 2). Flowers of this plant are short, dense and panicled spikes (Backer and Bakhuizen, 1963). The flowers are yellow in colour (Figure 3).

Figure 1. The shrubs of *S. crispus* in an herbal garden of Health Campus, Universiti Sains Malaysia, Malaysia.

Figure 2. The leaves of *S. crispus*.

Figure 3. *S. crispus* flowers.

Of late, there has been a few products are available in the health-food market that commercialize this plants as a healthy drinking. *S. crispus* products are available either in the form of raw crude powder (of leaves), as capsule, as an additive mixed with coffee or as a tea. These products can be found online in few websites such as *www.herba.com.my*, *http://www.bosland.com.my*, *www.detox-can.com* and so on. *S. crispus* tea is the most products that we can find online.

To our knowledge, till date, no particular review is available on *S. crispus* plant or on its products. Therefore, the present review is aimed to compile an up-to-date and comprehensive review of *S. crispus* that covers its ethnomedicinal uses, phytochemical

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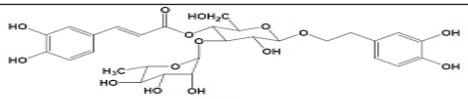
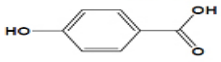
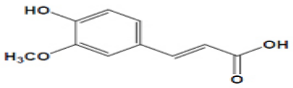
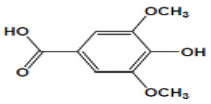
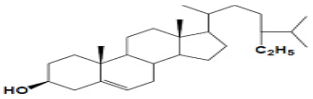
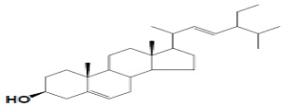
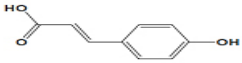
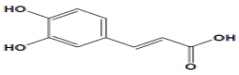
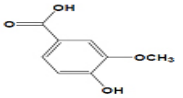
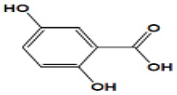
Table 1. Chemical constituents isolated from *S. crispus* according to its plant part and group of compounds.

| Name of compound | Structure | Group of compound | Plant part | Reference(s) |
|---|---|---|---|---|
| Verbascoside |  | Ester glycoside | Leaves | Ahmed, 1999, Soediro *et al.*, 1983 |
| ρ-hydroxy benzoic acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| Ferulic acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| Syringic acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| β-sitosterol |  | Phytosterols | Leaves | Asmah *et al.*, 2006a |
| Stigmasterol |  | Phytosterols | Leaves | Afrizal, 2008, Asmah *et al.*, 2006a |
| ρ-caumeric acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| Caffeic acid |  | Phenolic acid | Leaves | Soediro *et al.*,1987 |
| Vanilic acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| Gentisic acid |  | Phenolic acid | Leaves | Soediro *et al.*, 1987 |
| Tritriacontane |  | alkane | Leaves | Afrizal, 2008 |

contents and scientifically proven pharmacological and toxicological properties. Hopefully the information provided in this review will be useful and applicable for future research works aiming towards exploiting the plants nutraceutical potentials.

*Traditional Uses*

Traditionally, this plant is used for antidiabetic, antilytic, laxative, anticancer and as a diuretic agent (Sunarto, 1977; Perry and Metzger, 1980). This plant has many cystoliths of calcium carbonate and an infusion is mildly alkaline (Perry and Metzger, 1980). The high content of this calcium carbonate make this boiled water of this plant become mildly alkaline and function in ease of urination (Noraida, 2005).

Samuel *et al.* (2010) reported that orang asli in Kampung Bawong, Perak of West Malaysia masticated and swallowed the fresh leaves of this plant to enhance the immune system. A survey of the Malay herbal medicine in the Gemencheh settlement, Negeri Sembilan state, Malaysia, revealed the application of *S. crispus* to treat kidney stones by placed the heated leaves on the hips (Ong and Norzalina, 1999).

*Phytochemical constituents*

Various phytochemical groups and constituents have been identified in *S. crispus* (Tables 1). Study by Maznah *et al*. (2000) showed that *S. crispus* contains polyphenols, catechins, alkaloids, caffeine, tannins, vitamins (C, B1 and B2) and also high mineral content including potassium (51%), calcium (24%), sodium (13%), iron (1%) and phosphorus (1%). Ahmed (1999) reported the isolation of verbascoside from methanol extract. Soediro *et al.* (1987) isolated ester glycoside compounds of verbascoside (Soediro *et al.*, 1983) and 7 phenolic acid (p-hydroxy benzoic, p-coumeric, caffeic, vanilic, gentisic, ferulic and syringic). Asmah *et al.* (2006a) successfully isolated bioactive components: stigmasterol and β-sitosterol.

Liza *et al.* (2010) have identified eight flavonoid compounds from the leaves of *S. crispus* by using HPLC. The identified compound included (+)-catechin, (−)-epicatechin, rutin, myricetin, luteolin, apigenin, naringenin and kaempferol. Muslim *et al.* (2010) examined phytoconstituents of methanolic and aqueous extracts of *S. crispus* dried leaves using GC-TOF mass spectroscopy

and identified 32 compounds for methanolic extract and 21 compounds in aqueous extract. The identified components in methanolic extract included 3-octadedecyne, α-sitosterol, campesterol, hexadecanoic acid,methylester, lupeol, phytol and stigmasterol while in aqueous extract included 3,5-dithiahexanol,5,5-dioxide, cyclobutanol, hydrazine carboxamide, monoethanolamine, n-prophyl acetate and undecane. Afrizal (2008) reported the presence of secondary metabolites in *S. crispus* leaves included α-sitosterol, campesterol, phytol and stigmasterol.

Even though various types of chemical compounds have been identified from *S. crispus*, research reports on isolated compound and the bioactivity and the mechanism of action of the isolated compounds are limited. Additionally the effects of these compounds on the ailments like cancer, HIV, blood pressure, cardio-vascular disease and others, need to be investigated in detail.

*Volatiles*

Each plant species have volatile compounds which possess their own characteristic smell. This might be useful from differentiating the plant from other closely related subspecies. Asmah *et al*. (2006b) analysed essential oil obtained from hydrodistilled (at 100°C for 6 h) of fresh leaves of *S. crispus* by using GC–MS and revealed at least 28 compounds (Table 2). Further, the major volatile compounds identified included: phytol (46.01%), alpha cadinol (3.47%), tau-murolol (2.49%), iedol (1.81%) and eugenol (1.08%).

However, additional works are warranted to search for possible biological activities of these volatile compounds including oils and also the possibilities for their commercial exploitation.

*Pharmacology properties*

Even though several traditional uses of *S. crispus* are recognized, a scientific validity and supporting evidence is a pre-essential for commercial exploitation. In the preceding text some of the available reports pertaining towards the pharmacological potential of the plant extracts are being discussed. Tables 3 provide an overview of some important works on the pharmacological properties, the isolated chemical compounds and their activity undertaken on the *S. crispus* plant.

*Anti-diabetic properties*

Traditionally, daily consumption of *S. crispus* leaves has been believed to control blood sugar levels. However, the scientific information available on this aspect relevant to human model is very limited, indicating the need for elaborative research works to be undertaken in the near future.

Table 2. Volatile compound of essential oil from *S. crispus* leaf.

| Peak | Retention Time | Substance | (%) |
|---|---|---|---|
| 1 | 10.830 | 2,3-dihydrobenzofuran | 1.68 |
| 2 | 16.216 | Megastigmatrienone | 1.21 |
| 3 | 16.865 | Unknown | 1.73 |
| 4 | 17.108 | Alpha-cadinol | 3.47 |
| 5 | 17.267 | Tau-murolol | 2.49 |
| 6 | 17.342 | Unknown | 1.21 |
| 7 | 17.392 | Ledol | 1.81 |
| 8 | 17.525 | 1-Naphtalenol | 1.97 |
| 9 | 17.992 | Eugenol | 1.08 |
| 10 | 19.133 | 2-Undecanone | 5.84 |
| 11 | 19.308 | Phenol | 3.07 |
| 12 | 20.042 | 2-hexy,1-decanol | 3.69 |
| 13 | 20.283 | Isophytol | 0.96 |
| 14 | 20.800 | Nonadecanoic acid | 2.10 |
| 15 | 20.918 | 9,17-Octadecadienal | 2.34 |
| 16 | 22.000 | Hexyl octyl ether | 1.77 |
| 17 | 22.082 | Phytol | 46.01 |
| 18 | 22.443 | Tetradecanal | 0.87 |
| 19 | 22.873 | 2,6,10-trimethyl pentadecane | 0.93 |
| 20 | 23.839 | Eicosane | 1.10 |
| 21 | 24.071 | 13-tetradece-11-yn-1-ol | 1.02 |
| 22 | 24.768 | Heptadecane | 1.26 |
| 23 | 25.691 | Tridecy iodide | 1.70 |
| 24 | 26.243 | Di-n-octyl phthalate | 2.62 |
| 25 | 26.687 | Tetratetracontane | 1.45 |
| 26 | 27.804 | Octacosane | 1.88 |
| 27 | 30.614 | Pentadecane | 3.00 |
| 28 | 34.590 | Heptacosane | 1.73 |

Table 3. An overview of some important works on the pharmacological properties of *S. crispus* plant.

| Plant part used | Activity | Reference |
|---|---|---|
| **Leaves** | Anti-diabetic | Mohd Fadzelly *et al.*, 2006a, Norfarizan-Hanoon *et al.*, 2009a |
| **Leaves** | Wound healing | Al-Henhena *et al.*, 2011, Norfarizan-Hanoon *et al.*, 2009b |
| **Leaves** | Antimicrobial | Ahmed, 1999, Muskhazli *et al.*, 2009 |
| **Leaves** | Antioxidant | Asmah *et al.*, 2006b, Mohd Fadzelly *et al.*, 2006b, Muslim *et al.*, 2010, Norfarizan-Hanoon *et al.*, 2009a, Maznah *et al.*, 2000, Suhailah *et al.*, 2011, Mohammad Iqbal *et al.*, 2010, Maznah *et al.*, 2012 |
| **Leaves** | Anticancer | Asmah *et al.*, 2006a, Asmah *et al.*, 2006b, Mohd Fadzelly *et al*,. 2006b, Chong *et al.*, 2012, Muslim *et al.*, 2010, Fauziah *et al.*, 2005, Suhailah *et al.*, 2011, Hanachi *et al.*, 2008, Kusumoto *et al.*, 1992, Nik Soriani *et al.*, 2010, Suherman *et al.*, 2001, Suherman *et al.*, 2004, Susi *et al.*, 2001, Susi *et al.*, 2007a, Susi *et al.*, 2007b, Yogespiriya *et al.*, 2005 |
| **Leaves** | Anti-ulcerogenic | Mahmood *et al.*, 2011 |
| **Leaves** | Toxicology | Al-Henhena *et al.*, 2011, Lim *et al.*, 2012, Norfarizan-Hanoon *et al.*, 2009c, Norfarizan-Hanoon *et al.*, 2012 |

Mohd Fadzelly *et al.* (2006a) have reported the hyperglycaemic activity of *S. crispus* in the rat model system. They screened the aqueous extracts of fermented and unfermented of *S. crispus* tea to determine the blood glucose lowering effect in normal and streptozotocin-induced hyperglycaemic rats for 21 days. From the results obtained, the administration of hot water extracts of both fermented and unfermented *S. crispus* tea revealed positive results

in hyperglyacaemic rats. *S. crispus* unfermented tea also decreases the level of glucose in normal rats but not fermented tea. Both fermented and unfermented *S. crispus* tea showed to improve lipid profile.

Norfarizan-Hanoon *et al.* (2009a) have reported the effect of *S. crispus* juice on glucose, lipid profile, glutathione peroxidase and superoxide dismutase in normal and streptozotocin-induced diabetic male and female albino Sprague Dawley rats. They conducted the study on normal and streptozotocin-induced diabetic male and female Sprague Dawley rats fed with basal diet and *S. crispus* juice with different doses 1.0, 1.5 and 2.0 mL $kg^{-1}$ b.wt. for 30 days. The results showed that significant ($p < 0.05$) decrease in serum glucose levels in male and female diabetic and normal rats with treated *S. crispus* juice (1.0, 1.5 and 2.0 mL $kg^{-1}$ b.wt.). Cholesterol and triglyceride level significantly ($p < 0.05$) decreased in diabetic rats treated with 1.0, 1.5 and 2.0 mL $kg^{-1}$ b.wt. of *S. crispus* juice. Cholesterol, triglyceride and LDL-cholesterol level showed reduction in treated male and female normal rats. HDL-cholesterol showed the increasing but not significant ($p < 0.05$) difference in treated diabetic and normal male and female rats. Glutathione peroxidase and superoxide dismutase activities significantly ($p < 0.05$) increased in treated diabetic and normal male and female rats. Thus, the administration of *S. crispus* juice possesses antihyperglycemic, hypolipidemic and antioxidant effect in normal and streptozotocin-induced diabetic rats.

*Wound healing properties*

Research into the role of plant extracts in wound healing especially for diabetic patients assumes importance. However, scientific information available for this aspect is very limited.

Norfarizan-Hanoon *et al.* (2009b) have reported the effect of *S. crispus* juice on wound in normal and streptozotocin-induced rats. They screened the juice extracts (70, 105 and 140 mg/kg b.wt) of fresh leaves of *S. crispus* to determine the wound healing effect in normal and streptozotocin-induced hyperglycaemic rats by monitored the healing of 2 cm linear incisions created on the back of each rat and measuring the length of the wounds daily. From the results obtained, *S. crispus* juice enhanced wound healing where the percentage of wound healing at day 3 and 7 in the treated group especially treated with 140 mg/kg b.wt. of *S. crispus* juice in diabetic and normal rat is significant increase ($p < 0.05$) compared with the control.

Al-Henhena *et al.* (2011) evaluated the effects of topical application of ethanol extract of *S. crispus* leaf on the rate of wound healing closure and histology of healed wound. Four groups of male Sprague Dawley rats, all were experimentally wound in the posterior neck area. An area of uniform wound 2 cm in diameter using circular stamp, was excised from the nape of the dorsal neck of all rats with the aid of round seal. The animal groups were topically treated respectively with 0.2 ml of each vehicle (gum acacia), intrasite gel (reference control), 100 and 200 mg/ml of ethanol extract. Their results showed that wound dressed with leaf extract and intrasite gel-treated group significantly healed earlier than those treated with vehicle, which 100 mg/ml *S. crispus* extract, 200 mg/ml *S. crispus* extract and intrasit gel required healing time of 14.80, 13.00 and 13.17 days to heal the wound. The histological analysis of healed wounds dresses with leaf extract showed comparatively less scar width at wound closure and healed wound contained less inflammatory cells and more collagen with angiogenesis compared to wounds dressed with vehicle. This study clearly suggested that wounds dressed with leaf extract significantly enhanced the acceleration of wound healing enclosure in rats, and this was ascertain by histological study.

*Antimicrobial activities*

Exploration of the possibilities of any herbal plant extract to be a potential agent against pathogenic microbes assumes importance from a consumer's point of view.

Muskhazli *et al.* (2009) evaluated the antibacterial activity of six methanolic crudes extract from six Malaysian medicinal plants including *S. crispus*, against *Bacillus cereus*. Different concentrations of the methanol solvent crude extract from selected plants were used (1, 2, 4, 6, 8, 10, 15, and 20 mg/ml) and the diameter of *B. cereus* growth inhibition zone was measured at every 24 hours for 5 days. From their study, they reported that the crude extract of *S. crispus* was active on *B. cereus* by showing the largest mean of diameter inhibition zones at the concentration of 20 mg/ml. *S. crispus* crude extract also showed the Minimal Inhibition Concentration (MIC) value at 2 mg/ml, while the Minimal Bactericidal Concentration (MBC) at much higher concentration with the MBC values at 6 mg/ml.

Ahmed (1999) tested the crude extract of the leaves of different solvents (methanol and water) and butanol fractions for antibacterial activities against both gram positive and gram negative bacteria such as *Staphylopoccus aureus*, *Streptococcus faecalis*, *Vibrio cholerae* and *Pseudorrwnas aeruginosa*. From the result obtained, the extracts and the fractions were effective against the two strains of bacteria. Further,

he isolated verbascoside from the plant and tested it for antibacterial activity against three types of bacteria such as *Staphylococcus aureus*, *Salrrwnella typhi* and *Pseudorrwnas aeruginosa* and compared with other drugs like Penicillin (10 µg/ disc), Erythromycin (15 µg/disc), and Tetracycline (30 µg/ml). The results showed that the compound was very effective as an antibacterial agent. *In vivo* testing also showed good effect against *Staphylococcus aureus* and *Salrrwnella typhi* and its effective dose against both organisms was calculated to be (38. 481 mg/kg and (35.539 mg/ kg), respectively.

However, based on the availability of the reports, there is still a wide gap in looking for the antibacterial activities against other pathogenic microorganisms such as *Listeria monocytogenes* or other pathogenic fungi. Hence, further studies in this view are deserved.

*Antioxidant properties*

A few authors have shown that *S. crispus* is endowed with strong *in vitro* and *in vivo* antioxidant properties. Mohd Fadzelly *et al.* (2006b) have reported antioxidant activity of various types of *S. crispus* tea. They screened the fermented and unfermented of *S. crispus* tea from young and old leaves for the possible antioxidant activity *in vitro* using FRAP (Ferric Reducing/Antioxidant Power) and DPPH free radical scavenging assay and compared it with commercial tea (green and black tea). Their study revealed that both methods showed the same trend for antioxidant activities with green tea possessed the highest antioxidant activity, followed by black tea, *S. crispus* unfermented tea (old leaves), *S. crispus* unfermented tea (young leaves), *S. crispus* fermented tea (old leaves) and *S. crispus* fermented tea (young leaves).

Maznah *et al*. (2000) evaluated the total antioxidant activity of dry leaves of *S. crispus* using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods and compared their results to the previously reported results of Yerbamate, green tea, black tea and Indian tea. From their study, they reported that the extract of *S. crispus* showed highest antioxidant activity (96%) followed by Yerbamate (82%) and vitamin E (control) (76%).

Muslim *et al.* (2010) have investigated the antioxidant activity of methanolic and aqueous extracts of *S. crispus* using DPPH free radical, xanthine oxidase activity and β-carotene-linoleate model system. In DPPH assay, 1000 µg/ml of extracts was used with gallic acid, ascorbic acid, quercetin and Butylated Hydroxyl Anisole (BHA) as the control. Meanwhile, in xanthine oxidase assay, 100 µg/ml of extracts were used with xanthine substrate solution as the control. In β-carotene-linoleate model system assay, 200 ppm extracts were used with quercetin, BHA and BHT as the control. *S. crispus* extracts displayed very strong inhibitory activity towards xanthine oxidase enzyme where at 100 µg/ml concentration, methanolic extract showed 90.28% and the aqueous extract exhibited 89.06% xanthine oxidase inhibition. However, the extracts showed moderate antioxidant properties which is evidenced by the quenching of DPPH free radical and preventing the bleaching of β-carotene by linoleic acid. The $EC_{50}$ value of controls: gallic acid, quercetin, BHA and ascorbic acid in the DPPH assay displayed potent DPPH free radical scavenging activity, which produced $EC_{50}$ of 12.6, 15.3, 21.9 and 25.5 µg/ml, respectively. In β-carotene-linoleic acid assay, at the concentration of 200 ppm, were found the methanolic and aqueous extract to produce 1.51% and 0.91% inhibition in comparison to BHA and BHT, which produce about 95.84% and 95.12% inhibition, respectively.

Asmah *et al.* (2006b) have investigated the antioxidant activity of essential oil of *S. crispus* and *Lawsonia inermes* fresh leaves using FTC and TBA methods. The results showed that the S. crispus oil has higher antioxidant activity compared to α-tocopherol (standard) but lower than *L. inermes*.

Suhailah *et al.* (2011) evaluated five aqueous and five ethanolic extracts from five traditional Malaysian plants including *S. crispus* for their antioxidant properties and total phenolic content. Antioxidant activities were evaluated using DPPH and FRAP assay with gallic acid used as standard positive control. Their results showed that for both assays (DPPH and FRAP), aqueous extracts of *S. crispus* (28.5% inhibition and 150.3 mmol/g, respectively) contain high antioxidant activities compared to ethanolic extracts (14.5% inhibition and 108 mmol/g, respectively). However, the extracts antioxidant activities were considered to be lowered than that of the gallic acid, which produced 88.8% inhibition for DPPH assay and 1216.67 mmol/g for FRAP assay. The findings exhibited a strong correlation between antioxidant activity and the total phenol contents.

*Anticancer activity*

Several reports are available on the possible inhibitory of cancer by the extracts of *S. crispus*. Mohd Fadzelly *et al.* (2006b) evaluated the fermented and unfermented of *S. crispus* tea from young and old leaves for its possible antiproliferative properties effect against hormone-dependent breast cancer cell lines (MCF-7) and hormone-independent breast cancer cell lines (MDA-MB-231). Their results indicated

that the hot water extract of *S. crispus* unfermented tea from old leaves and *S. crispus* fermented tea from old leaves displayed cytotoxicity effect on MCF-7 but not the MDA-MB-231 cell lines.

Methanolic and aqueous extract of the leaves of *S. crispus* was studied by Muslim *et al.* (2010) for the possible cytotoxicity effects against MCF-7, colon carcinoma (HCT 116), liver cancer cell lines (HepG2), non-small cell lung adenocarcinoma (NCI-H23), human breast ductal carcinoma (T-47D) and normal colonic fibroblast cell line (CCD-18Co). The cell proliferation assay was evaluated using MTT assay and the inhibitory effect of the extracts on angiogenesis was evaluated using *ex vivo* rat aortic ring assay. 150, 100, 80, 60, 40, 20, 10 and 5 µg/ml of extracts was tested in this study and 600 ng/ml of vincristine was used as the positive control. Their results showed that the aqueous extract was nontoxic towards all cell lines used ($IC_{50}$ > 200 µg/ml), while the methanolic extract exhibited cytotoxic response towards the T-47D and MCF-7 cells which produced $IC_{50}$ value of 121.53 and 160.16 µg/ml, respectively. Both extracts showed detectable anti-angiogenic activity.

Asmah *et al.* (2006b) have reported that essential oil of *S. crispus* fresh leaves did not give any cytotoxic value against all the cell lines tested (MDA-MB-231, HepG2, colon cancer cell lines (CaCo-2), MCF-7 and Chang liver cell lines).

Suhailah *et al.* (2011) evaluated five aqueous and five ethanolic extracts from five traditional Malaysian plants including *S. crispus* for their cytotoxic activity against a normal human lung fibroblast cell line (Hs888Lu). The assay was carried out using Promega Cell Titer 96 $AQ_{ueous}$ Non-Radioactive Cell Proliferation (MTS) assay in triplicates at different concentration of extracts ranging from 100 µg to 500 µg. From their result, all the plant extracts of *S. crispus* showed non-toxic effects against Hs888Lu.

Susi *et al.* (2007a) have conducted to study and compare the cytotoxic effects of leaves extract of two plants, *Lawsonia inermis* and *S. crispus*. The chloroform extract of these two plants were subjected to cytotoxic study on several kinds of cancer cell lines such as Caco-2, HepG2, MCF-7, MDA-MB-231 and Chang liver cell lines by using MTT assay. They reported that the extract of *S. crispus* is found to be cytotoxic with $IC_{50}$ of 25.1 µg/ml and 28 µg/ml on Caco-2 and HepG-2 cell lines, respectively. They also studied the cytotoxic mechanism by determined the effect of adding the extracts to the *c-myc* gene expression using RT-PCR and sequencing process methods. They concluded that the cytotoxic effect of this extract may be mediated by the down-regulation of *c-myc* expression.

In the same year, Susi *et al.* (2007b) investigated the mechanism of anticarcinogenic effect of *S. crispus* extract through apoptotic pathway. Two doses (20 and 30 µg/ ml) of *S. crispus* chloroform extracts were subjected to TUNEL assay. The TUNEL assay was carried out by using Apoptosis Detection System, Fluorescein. The samples were analysed immediately under a fluorescence microscope using a standard fluorescein filter set to view the green fluorescence of fluorescein (FITC) at 520 ± 20 nm and red fluorescence of propidium iodide (PI) at > 620 nm. Confocal Laser Scanning Microscope (CLSM) was also used to obtain the better results. Exposure of HepG-2 cells to *S. crispus* extract resulted in induction of apoptosis in a dose-dependent manner.

Asmah *et al*. (2006a) evaluated the different type of extracts obtained from three types of extraction methods (catechin extraction, single solvent extraction using ethanol, methanol and chloroform and stepwise extraction using hexane, chloroform, ethyl acetate and methanol) and also bioactive compounds (β-sitosterol and stigmasterol) isolated from leaves of *S. crispus* for its possible antiproliferative activities using MTT assay against HepG-2, MCF-7, MDA-MB-231, Caco-2 and Chang liver cell lines. Their results indicated methanolic extracts showed the strongest cytotoxic effect on Caco-2, followed by MDA-MB-231 and HepG-2 with $IC_{50}$ values of 22.3, 27.2 and 29.3 µg/ml, respectively. The chloroform extract also have cytotoxic effect against Caco-2 and HepG-2 with $IC_{50}$ values of 25.1 and 28.0 µg/ml, respectively. They also indicated the β-sitosterol displayed cytotoxic properties against Caco-2, HepG-2 and MCF-7 with $IC_{50}$ values of 20.0, 53.0 and 71.2 µM, respectively. Meanwhile, stigmasterol inhibited the proliferation of Caco-2, HepG-2, MCF-7 and MDA-MBB-231 with $IC_{50}$ values of 132.5, 182.5, 156.0 and 185.9 µM. There was no significant cytotoxic effect on normal cell line in all samples tested.

Bioactivity-guided fractionation of the dichloromethane extract of the leaves of *S. crispus* was studied by Nik Soriani *et al.* (2010) for the possible anticancer activities using LDH Cytotoxicity Detection Kit, and the anticancer activity of one of the bioactive sub-fractions, SC/D-F9, was further analysed in breast and prostate cancer cell lines. They also determined apoptosis using Annexin V antibody and propidium iodide staining and analysed by fluorescence microscopy and flow cytometry, while caspase 3/7 activity was determined using FLICA caspase inhibitor and analysed by fluorescence microscopy. Their results revealed that selected sub-fractions of the dichloromethane extract induced

death of MCF-7, MDA-MB-231, PC-3 and DU-145 cells and the sub-fraction SC/D-F9, consistently killed breast and prostate cancer cell lines with low $EC_{50}$ values but are non-cytotoxic to the normal breast epithelial cell line, MCF-10A. They also indicated that SC/D-F9 displayed relatively higher cytotoxicity compared to tamoxifen, paclitaxel, docetaxel and doxorubicin. Cell death induced by SC/D-F9 occurred via apoptosis with the involvement of caspase 3 and/or 7.

Susi *et al.* (2001) investigated the possible cancer suppressive effect of leaf extracts *S. crispus* collected from Malaysia due to its non-nutritional and antioxidant activities. Cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for the fifth fraction of methanolic extract of *S. crispus* (FR-5) against different cancer cell lines. Solid Phase Extraction Vac Master was used for fractionation with $C_8$ as an isolute. The fifth fraction of this methanolic extract (FR-5) showed better cytotoxic effects with $IC_{50}$ values of 66.75, 83.75, 39.25 and 84.50 µg/ml against Caco-2, HepG2, MCF-7 and MDA-MB-231, respectively. This fraction did not show any cytotoxic effect against Chang liver cell line. These data indicated that FR-5 possessed a potent cytotoxic effect and the active compound of this fraction needed further study.

Chong *et al.* (2012) investigated chemoprevention activities of ethanol extract of *S. crispus* towards induction of apoptosis of MCF-7. *S. crispus* was able to reduce cell viability and proliferation in MTT and BrdU assays. Both cell cycle progression and Tunel assay suggested that $IC_{50}$ of *S. crispus* ethanol extract ($IC_{50}$ = 30 µg/ml) induced sub-G1 cell cycle phase, and DNA fragmentation. On the other hand, translocation of mitochondria cytochrome c release, induction of caspase 3/7 and p53 while suppress XIAP on treated MCF-7 cell were also observed in this study. *S. crispus* ethanol extract induced apoptosis and DNA fragmentation on hormone dependent breast cancer cell line MCF-7 via mitochondria dependent p53 apoptosis pathway.

Maznah *et al.* (2012) investigated the anticancer activity of 28 extracts from different parts of four edible medicinal plants indigenous to Malaysia, which included *S. crispus*, using the MTS assay, on four human cancer cell lines: colon (HT-29), breast (MCF-7), prostate (DU-145) and lung (H460). However, for *S. crispus* extracts, they only test it on HT-29 cell lines. The flowers and leaves of *S. crispus* were sequentially extracted with hexane, dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH). In terms of $IC_{50}$, the value recorded for treatment of S. crispus extracts against HT-29 cell lines for EA and MeOH extracts of leaves were 70.2 and 59.0 µg/ml, while for DCM and EA extracts of flowers were 90.3 and 42.0 µg/ml, respectively. No activity was recorded for hexane and DCM extract of leaves and hexane and MeOH extracts of flowers.

Several reports are available on the various ways of monitoring the carcinogenic process. Suherman *et al*. (2001) examined the possible cancer suppressive effect of various concentrations of *S. crispus* extracts from Selangor, Malaysia on hepatocarcinogenesis and the effect of 2-acetylaminofluorene (AAF) and diethylnitrosamine (DEN) in the kidney. A total of 55 male Sprague Dawley rats (*Rattus norwegicus*, 150-200 g or 6-8 weeks) were randomly distributed into 11 groups; normal, normal + *S. crispus* extract (SCE) 1%, normal + SCE 2.5%, normal + SCE 5%, cancer, cancer + SCE 1%, cancer + SCE 2.5%, cancer + SCE 5%, cancer + glycyrrhizin 1%, cancer + glycyrrhizin 2.5% and cancer + glycyrrhizin 5%. The cancer groups were treated with 200 mg/kg DEN as the initiator and 0.02% of AAF as the promoter without partial hepatectomy. The animals were sacrificed by cervical dislocation after 14 weeks. All the rats were dissected and the kidneys were collected. Application of histological evaluation was used in this *in vivo* study. Light microscope was used to determine the severity of kidney lesion. Lesion scoring was done on all the kidney sections and the mean lesion score was determined. The result showed that the lesion score of kidney in cancer-treated rats with SCE 5% was better in suppressing renal lesion. However, glycyrrhizin was found to be better in suppressing pathological changes in the kidney. The significance and the outcome of this study could be associated with prognosis that is very important in medical treatment management.Their result showed that the lesion score of kidney in cancer-treated rats with *S. crispus* 5% was better in suppressing renal lesion but glycyrrhizin was found to be better in suppressing pathological changes in the kidney.

Further, Hanachi *et al.* (2008) investigated the anticancer potency of *S. crispus* extract on DEN and AAF induced HCC with special attention to hepatic drug metabolism and investigated the effect of *S. crispus* on preneoplastic marker enzyme activity specifically of microsomal aniline hydroxylase (AH) activity and lesion scoring in rats treated with DEN and AAF and controls. Thirty male Sprague Dawley rats were divided to six equal number groups. In the first three groups, hepatocellular carcinoma was induced with diethylnitrosamine and acetylaminofluorene and three groups in each branch were randomly assigned to receive 5% w/v of *S.*

*crispus* extract, glycyrrhizin or no treatment. The rats were sacrificed after 12 weeks of treatment. Lesion scoring analysis and Aniline hydroxylase assays were performed as outcome measures. The obtained results have shown a significant, increase ($p < 0.05$) of liver microsome AH in cancer group rats after 12 weeks. Treatment with glycyrrhizin caused decrease in liver AH activity compared to control group. Meanwhile, treatment with *S. crispus* caused overall decrease in liver AH activity almost near to control group. Microscopic observation of the lesion score during hepatocarcinogenesis revealed that cells of cancer group without treatment were severely necrotic at week 12. *S. crispus* treatment reduced the severity in cancer group rats at week 12. Fauziah *et al.* (2005) also determined the anticancer potency of *S. crispus* extract on DEN and AAF induced hepatocellular carcinoma (HCC) with special attention to hepatic drug metabolism and to investigate the effect of *S. crispus* on preneoplastic marker enzyme activity specifically of microsomal aniline hydroxylase (AH) activity and lesion scoring in rats treated with DEN and AAF and controls. From their study, *S. crispus* only ameliorated the cancer incidence in the liver, however did not fully recover the liver tumour similar to the normal cells.

Suherman *et al.* (2005) evaluated the effect of administration of *S. crispus* extract on the histology and tumour marker enzymes, glutathione S-transferase (GST) and uranyl diphosphate glucoronyl transferase (UDPGT) in rat liver induced with hepatocarcinogen DEN and AAF. 1, 2.5, 5 and 7.5% (w/v) of *S. crispus* extract were used and compared with Glycyrrhizin (commercial anticancer drug used mainly for liver). The severity of the liver cell dysplasia was decreased by *S. crispus* extract treatment group as compared to glycyrrhizin. *S. crispus* also did not affect the normal organization in the liver. These findings suggested that the supplementation of *S. crispus* on DEN/AAF rats reduced the severity of hepatocarcinogenesis.

Meanwhile, Suherman *et al.* (2004) also evaluated the effect of *S. crispus* (1, 2.5, 5 and 7.5% (w/v)) by identifying activities of liver and plasma γ-glitamyl transpeptidase (GGT) and alkaline phosphatise (ALP) and also glutathione (GSH) concentration in hepatocarcinogenesis induced by DEN and AAF in rats. The administration of *S. crispus* to induced cancer rats decreased the microsomal GGT. These findings suggested that the supplementation of *S. crispus* on DEN/AAF rats reduced the severity of hepatocarcinogenesis by reducing liver GGT and ALP activities and also the levels of GSH.

Yogespiriya *et al.* (2005) investigated the effectiveness of 5% (w/v) *S. crispus* extract on rat liver during chemically induced hepatocarcinogenesis. Histological evaluation of liver was conducted in order to observe the cellular and morphological changes during hepatocarcinogenesis threated with *S. crispus*. Their results indicated that a certain grade of inflammation or necrosis at portal and lobular region and stages of fibrosis during hepatocarcinogenesis was successfully reduced after administration of *S. crispus* extract but these changes did not fully recovered by supplementation of *S. crispus* to normal histological features of liver due to a short experimental duration and also the supplementation of this extract to normal rat did not show any changes in normal hepatocytes.

Mohammad Iqbal *et al.* (2010) evaluated the effect of *S. crispus* aqueous extract for possible protection against lipid peroxidation and DNA damage induced by iron nitrilotriacetate (Fe-NTA) and hydrogen peroxide ($H_2O2$). They found that the incubation of post mitochondrial supernatant and/or calf thymus DNA with $H_2O_2$ (40 mM) in the presence of Fe-NTA (0.1 mM) induces lipid peroxidation and DNA damage to about 2.3-fold and 2.9-fold, respectively, as compared to control ($P < 0.05$). In lipid peroxidation protection studies, *S. crispus* treatment showed a dose dependent inhibition (45–53% inhibition, $P < 0.05$) of Fe-NTA and $H_2O_2$ induced lipid peroxidation. Similarly, in DNA damage protection studies, *S. crispus* treatment also showed a dose-dependent inhibition (18–30% inhibition, $P < 0.05$) of DNA damage. The protection was closely related to the content of phenolic compounds as evident by *S. crispus* extract showing the value of 124.48 mg/g total phenolics expressed as gallic acid equivalent (GAE, mg/g of extract). From their study, they concluded that *S. crispus* could be used as a potent chemopreventive agent and possesses the potential to be used to treat or prevent degenerative diseases where oxidative stress is implicated.

Kusumoto *et al.* (1992) investigated the methanol and water extracts of 30 Indonesian medicinal plants, including *S. crispus* for inhibitory activity on avian myeloblastosis virus (AMV)-reverse transcriptase (RT). Two kinds of template-primers, $(rA)_n$-$(dT)_{12-18}$ and $(rC)_n$-$(dG)_{12-18}$ were examined for polymerization reaction but the inhibitory activity was stronger in the $(rC)_n$-$(dG)_{12-18}$ directed AMV-RT reaction in most of the samples. In the poly(rA)-oligo(dT) directed reaction, the $IC_{50}$ of water extract of S. crispus higher than methanol extract, with 100 and 820 µg/ml, respectively. In the $(rC)_n$- $(dG)_{12-18}$ directed reaction, the extracts showed potent inhibition ( > 85%) at 0.5 mg/ml. Further, the RT reaction was carried out in a reaction mixture supplemented with 0.5mg/mL of bovine serum albumin (BSA) to see whether the

substances bind unselectively to proteins or not. So, in this experiment, the RT inhibitory activities of the water extract of *S. crispus* were not changed in the presence of BSA. Thus, the water extract of *S. crispus* inhibit the proliferation of retroviruses; an agent in viral diseases such as as immunodeficiency syndrome (AIDS) and adult T-cell leukaemia.

*Anti-ulcerogenic*

Mahmood *et al.* (2011) evaluated the anti-ulcerogenic activity of *S. crispus* leaf extract against ethanol-induced mucosal injury in rats. In their study, five groups of Sprague Dawley rats were pre-treated respectively with: vehicle, distilled water (ulcer control), omeprazole (20 mg $kg^{-1}$, reference control), 250 mg/kg, 500 mg/kg and 1000 mg/kg *S. crispus* leaf extracts (experimental groups), 60 min prior to oral administration of absolute ethanol to generate gastric mucosal injury. Their results suggested that in rats given absolute ethanol gavages, pre-treatment with *S. crispus* leaf extract displayed gastroprotective activity. Rats pre-treated with *S. crispus* leaf extracts resulted in significantly dose-dependent reduction of gastric lesion formation accompanied by significant increase in gastric mucus production and pH of gastric fluid. Gastric protection was more prominent in 1000 mg/kg of *S. crispus*-treated group. Histology comparatively decreased gastric mucosal injury and inhibited edema and leukocytes infiltration in submucosal layer of stomach.

*Toxicology properties*

The lack of adequate supporting scientific information on the levels of safety, quality and toxicity associated is still a major problem in employing traditional medicines. To our knowledge, presently, there are no available reports on the purity (in terms of quality and safety) and on the side effects of long term use of the products prepared from the *S. crispus*.

Al-Henhena *et al.* (2009b) reported the acute toxicity of *S. crispus*. In their study, forty eight healthy Sprague Dawley rats (24 males and 24 females), divided into four groups labeled as vehicle (gum acacia in normal saline); 1, 2 and 5 g/kg of *S. crispus* in vehicle, respectively and were kept under observation for 14 days. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The animals were sacrificed on the 15$^{th}$ day. Their results showed that all the animals remained alive and did not manifest any significant visible of toxicity at these doses. Thus, clinical observations, blood biochemistry, haematology, and histopathology data did not show any significant differences between control and treated groups. They concluded that *S. crispus* orally administered to rats was safe and that no drug-related toxicity was detected even at the highest dose investigated.

Mahmood *et al.* (2011) studied the acute toxic to determine a safe dose for *S. crispus*. 48 healthy Sprague Dawley rats (24 males and 24 females) were divided equally into 4 groups labeled as vehicle (sterile distilled water); 1, 2 and 5 g/kg of *S. crispus* in vehicle, respectively. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any was observed over a period of 2 weeks. The animals were sacrificed on the 15$^{th}$ day. Hematological, serum biochemical and histological (liver and kidney) parameters were determined. After 14 days, all the animals remained alive and did not manifest any significant visible of toxicity at these doses. Thus, clinical observations, blood biochemistry, hematology, and histopathology data did not show any significant differences between control and treated groups. The results revealed that *S. crispus* orally administered to rats was safe and that no drug-related toxicity was detected even at the highest dose investigated.

Norfarizan-Hanoon *et al.* (2012) in one of their studies evaluated four different doses of *S. crispus* juice (700, 2100, 3500 and 4900 mg $kg^{-1}$ of body weight) administered orally to normal female and male Sprague Dawley rats on possible changes in various physical, behaviour, morphology and biochemical parameter. Rats were fed a single dose of *S. crispus* juice orally by gavage for day 1. For control (untreated) group, they were given commercial feed and plain water only. Their results showed the *S. crispus* juice to be safe at the maximum dose used in this study (4900 mg $kg^{-1}$ of body weight). No significant toxicity observed with respect to clinical parameters and organ morphology. Also, no significant changes were observed in the level of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and albumin.

Norfarizan-Hanoon *et al.* (2009c) reported safety effect of S. crispus juice in normal and streptezotocin-induced diabetic Sprague Dawley rats of both sexes. In their study, three levels of dosages (70, 105 and 140 mg/kg of body weight) were orally and repeatedly administered for 30 days. The rats were observed twice daily for mortality, signs of gross toxicity, behaviour changes and physical changes for 30 days after treatment. They also analysed the blood for haematology parameter and serum for determination

of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin and creatinine. Their results showed that *S. crispus* juice was found to be relatively safe as no mortality was noted even when the highest dose was used in normal rats. There were no changes observed with respect to the general behaviour, body weight and organ weight. The administration of *S. crispus* juice to normal group revealed insignificant change in liver and kidney functions but significant reduced of AST (group female and male rats with administration 140 mg/kg b.wt. of *S. crispus* juice), ALT (group female rats with administration 140 mg/kg b.wt. of *S. crispus* juice) and ALP (group female rats with administration 105 mg/kg b.wt. of *S. crispus* juice). In streptezotocin-induced diabetes, the blood glucose increased with increased in serum AST, ALT, ALP and creatinine. After 30 days of treatment with *S. crispus* juice, the AST, ALT, ALP and creatinine were reduced.

Lim *et al.* (2012) examined the oral toxicity of repeated dosing of *S. crispus* ethanol leaves extract on the liver and kidney functions in Sprague Dawley rats. 20 young female rats were assigned equally into four groups. The first group served as control, while the second, third and fourth groups were orally treated with a single dose daily with 150, 300 and 600 mg/kg of *S. crispus* ethanol leaves extract for 14 day consecutively. The body weight changes, food consumptions and water intake were recorded. Serum biochemical parameters (AST, ALT, ALP, creatinine and urea) were determined at day 15. The results showed that 14 day administration of *S. crispus* ethanol leaves extract did not cause any adverse effects or lethality to the female rats. There were no significant changes in serum biochemical parameters, relative organs weights, body weights, food intake and water consumptions between the treatment groups and control. Furthermore, the *S. crispus* ethanol leaves extract was safe without affecting the liver and kidney functions in female rats.

## Conclusion

In this paper, we briefly summarized the *in vitro* and *in vivo* assays applied in the discovery of possibly new pharmacological and functional agents from *S. crispus*. In addition, various literatures related to the pharmacological investigation of *S. crispus* were reviewed to gather all information related to the ethnobotanical, phytochemical, pharmacological and toxicological properties of *S. crispus*. A significant number of *in vitro* and laboratory animal studies have provided significant evidences that *S. crispus* possesses adequate therapeutic potential and could be explored further for commercial purposes. However, as most of the scientific reports are based on animal experiments, human trials might throw more insight onto the long term safety concerns of consuming the plant extract. Further research works needs to be done to provide scientific base and look for the possible role of these plants extract to treat tumour, wound healing and also the possible role of acting as anti-HIV, anti-diabetic, antioxidant, anticancer and antibacterial in human models. Even though there are various types of bioactive compounds isolated and identified from *S. crispus* as highlighted in the phytochemical section, their contribution towards the plant claimed medicinal uses or demonstrated pharmacological activities were also not fully studied. Thus, the quest for new compounds from *S. crispus* with specific pharmacological activity remains unsolved. It is suggested that researches should be increased to isolate, identify, and collect the compounds from *S. crispus* so that their pharmacological potential could be investigated thoroughly. In conclusion, it is hoped that this paper will serve as an encouragement for others to further explore the pharmacological potentials of *S. crispus* with hope of developing it as a new therapeutic agents, nutraceuticals and functional foods as it is considered as one of the important herbs, particularly in the Malay folk medicine.

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